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Transcriptional regulatory regions of testis-specific *PGK2* defined in transgenic mice

(spermatogenesis/translational regulation/luciferase)

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ABSTRACT **The gene encoding testis-specific phosphoglycerate kinase 2 (PGK; ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) is expressed only in meiotic and haploid male germ cells. Transgenic mice containing an 8-kilobase human genomic *PGK2* gene express the human gene in a tissue-specific and developmentally regulated manner. To determine the nature and location of sequences controlling this expression, transgenic mice with various lengths of the human *PGK2* 5′ region fused to the chloramphenicol acetyltransferase (CAT) gene were analyzed for expression. A 323-base-pair region 5′ to the coding region was found to contain information essential for both tissue-specific and developmentally regulated expression of the CAT reporter gene. Transgenic mice containing a *PGK2*/luciferase-coding construct were compared with mice containing an equivalent CAT construct. Luciferase gene expression was also testis-specific and was more sensitive than CAT gene expression, but otherwise regulation of the two reporter genes was similar in the germ cells of transgenic mice. Translation of both *PGK2*/CAT and *PGK2*/luciferase fusion genes was seen concurrently with the first detectable transcripts.**

Spermatogenesis involves a complex developmental program including the progression of cells through mitotic, meiotic, and haploid stages (for reviews, see refs. 1 and 2). Mitotically dividing spermatogonia enter meiosis as spermatocytes and then go through two cell divisions to become round spermatids. These haploid cells undergo elongation, nuclear condensation, and other morphological changes, resulting in mature spermatozoa.

In the mouse, spermatogenesis is an ongoing process that begins soon after birth. Initial appearance of spermatogenic cell types occurs synchronously, and the sequence of events can be followed as a function of increasing age in a variety of strains of inbred mice (3); At day-9 or -10 *post partum*, spermatogonia first enter meiosis, by day-14 or -15 the first pachytene spermatocytes are seen, and by day-22, haploid round spermatids appear (3). The precise regulation of germ-cell differentiation requires a controlled program of stage-specific gene expression. To examine this process at the molecular level, the mechanisms involved in the transcriptional control of germ-cell-specific genes must be understood. A well-characterized gene product expressed during spermatogenesis and, therefore, a good candidate for molecular analysis is the testis-specific isozyme for the glycolytic enzyme phosphoglycerate kinase (PGK) (ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) (4–8).

In mammals PGK is encoded by two genes, *Pgk-1* and *Pgk-2* (*PGK1* and *PGK2* in human). *Pgk-1* is X-chromosome-linked and expressed in somatic cells (6, 9, 10), whereas the autosomal *Pgk-2* gene is expressed only in meiotic and haploid male germ cells (11, 12). *Pgk-2* transcription is first seen in pachytene spermatocytes, with message levels increasing during later stages of spermatogenesis (12–14). PGK-2 protein is not detected until 7 days later in haploid round spermatids (6–8) and is coincident with the appearance of *Pgk-2* message on polysomes (12), suggesting additional regulation at the translational level. Accordingly, analysis of cis-acting regulatory elements of *Pgk-2* may provide a basis for understanding the control of gene expression during spermatogenesis.

Human (13, 15, 16) and mouse (17) *Pgk-2* genomic sequences have been cloned, and both genes lack introns and share extensive homology. The sequences 5′ to the coding region are 66% homologous (17), and both genes exhibit similar promoter motifs, consisting of the consensus sequences for the SP1 transcription factor (GC box) and for nuclear factor 1 (CAAT box) (18, 19) (Fig. 1), rather than the TATA consensus sequence found adjacent to the start of the coding region in many genes (21). Given the reasonable extent of homology, the ability to compare mouse and human sequences for potential control elements, and the ease of detecting the human gene in a mouse background, we chose to define the extent of sequences required for appropriate expression of the human *PGK2* gene. We used transgenic mice to assay for cis-acting elements that control stage and cell type-specific gene expression.

Here we show that a human *PGK2* gene fragment is appropriately expressed in the mouse and that all or part of a 323-base-pair (bp) region 5′ to the coding region of the human *PGK2* gene is necessary to direct testis-specific expression of reporter genes.

MATERIALS AND METHODS

Gene Constructs. An 8-kilobase (kb) human genomic *PGK2* *Eco*RI fragment (Fig. 2*A*). that contains the *PGK2* coding sequence flanked by 2.5 kb 5′ and 4.25 kb 3′ to the gene was isolated for microinjection from a genomic clone generated by Szabo *et al.* (15). The *PGK2* 5′ region was isolated by subcloning a 2.2-kb *Sst* I fragment containing 1.4 kb of the 5′ region and the first 0.8 kb of the coding region into mp18, converting the sequences across the initiation codon into a *Hin*dIII site by site-directed mutagenesis, and isolating the resulting 1.4-kb *Hin*dIII fragment. The *PGK2*/chloramphenicol acetyltransferase (CAT) fusion gene was constructed by inserting the 1.4-kb fragment into the unique *Hin*dIII site of the plasmid pSVOCAT (22). This *PGK2*/CAT plasmid was digested with either *Xba* I, *Pst* I, or *Ava* II at the 5′ end and *Bam*HI at the 3′ end, and the resulting 3.1-kb, 2.2-kb, and 1.9-kb *PGK2*/CAT fragments (Fig. 2*B*) were each isolated for microinjection. The *PGK2*/luciferase fusion gene (Fig. 2*C*) was constructed by inserting the 1.4-kb *Hin*dIII 5′ region

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Abbreviations: PGK-2, phosphoglycerate kinase 2; CAT, chloramphenicol acetyltransferase; lu, luminometer unit.

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     PstI▼
-520 CTGCAGAGGT TTTTACATAT CAAAATGGTT AAGATTTGAC ATGAATGAGG
-470 TGTATGTAGG TTTGCGGGGG TGGGGGTGGA GTTCTTTTTG TTTTGTTTTT
-420 AATAATAAAA GCAACTGTTA ACCGAGCTGT GGGGTGGGGG CAAAAAGAGC
-370 CAGAAGCGGC GCACACCTCA GGACTATTCT TGTTTTTTTA GAACCATTCT
-320 TATTCTGGGG TTTCTTCACC TACCCAAGTC TCGCCTGAAG CCAGGTACAG
-270 CTCTATTCCA CTACATGACC CTCTGCCCAG GAAGTTGGAA TCTTCACCTA
                        AvaII▼
-220 GCAACACAGT TCAGATCGAG ATTGACAGGA CCATGAGCCA ATCACAAAGC
-170 TAGATTTGCT TTCAGTCTAA CAGTGGCCGT TGTGCTGGAG ACAGTAGGAG
-120 AAGAAAGGGG CGGGACAAGG GCAAAGGCGT TAGAAGTCAC CACCGACCCA
 -70 GCCCCTCAAC AGCAAGTTGG TTCTTCAGCA TTAAGATCCA GGTGTCAGCC
                        +1
 -20 TATGTCTTTA TATTGTCAAG ATG
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FIG. 1. The nucleotide sequence of the 5′ region of human *PGK2*. Nucleotides are numbered relative to the first nucleotide of the translation start codon (+1) and extend from the *Pst* I site at −515 bp relative to the initiation codon. The CAAT consensus sequence (position −184 bp) and the GC consensus sequence (position −114 bp) are boxed. Restriction sites used for transgenic constructs are indicated. The sequence was taken from McCarrey (20).

fragment into the unique *Hin*dIII site of the plasmid pSVOAL (23) and isolating the 3.5-kb *Xba* I–*Bam*HI fragment for microinjection.

Transgenic Mice. Each of the five constructs was microinjected into fertilized eggs from superovulated (C57BL6J/DBA2J) F_1 females mated to males of the same cross. Surviving eggs were then reimplanted into pseudopregnant (C57BL6J/DBA2J) F_1 females. Microinjection was performed essentially as described (24). Founder transgenic mice were identified by Southern blot hybridization using a 500-bp human *PGK2* ^{32}P-labeled probe that spans the initiation codon. Subsequent generations were identified by either dot-blot hybridization or amplification of a 780-bp *PGK2*/CAT fragment from 0.5 μg of genomic DNA using the polymerase chain reaction (25).

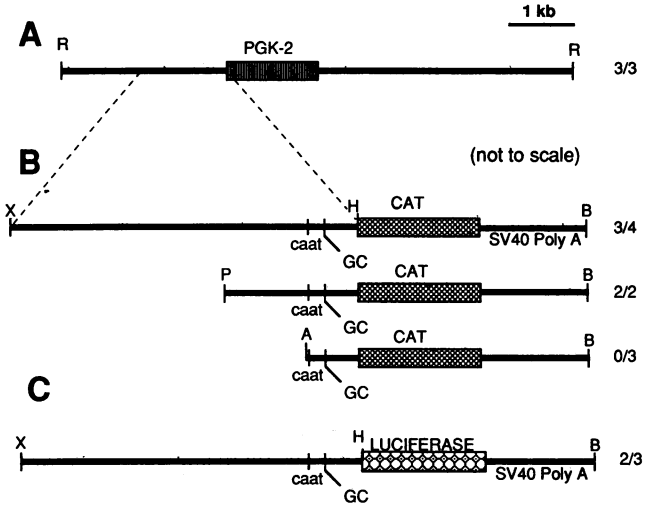

FIG. 2. Constructs injected into fertilized mouse embryos. (*A*) Structure of the genomic human *PGK2* gene *Eco*RI fragment. This 8-kb fragment contains 2.5 kb 5′ to the ATG initiation codon, a coding region of 1.25 kb contained within a single exon, and 4.25 kb 3′ to the gene. (*B*) *PGK2*/CAT fusion constructs. 1.4 kb of the human *PGK2* upstream region was placed into the *Hin*dIII site of the plasmid pSVOCAT, which contains the gene for CAT and simian virus 40 (SV40) poly(A) addition sequences. Restriction enzymes used to isolate each fragment are shown, as are positions of the CAAT consensus sequence (caat) and the SP1 binding-site consensus sequence (GC). (*C*) Structure of the *PGK2*/luciferase gene. The 1.4-kb promoter region was inserted into the *Hin*dIII site of pSVOAL that contains the luciferase-coding region and the simian virus 40 poly(A) addition sequences. The number of lines expressing the transgene versus the number of lines tested is shown at right. A, *Ava* II; B, *Bam*HI; *H*, *Hin*dIII; R, *Eco*RI; P, *Pst* I; X, *Xba* I.

RNA Analysis. Total RNA was extracted by tissue homogenization with 6 M urea/3 M LiCl, as described (26). Northern (RNA) analysis was done as described (27). The human message was analyzed by using the same probe as that used for Southern analysis. The endogenous mouse message was assayed using a ^{32}P-labeled probe from the 3′ untranslated region of the mouse *Pgk-2* gene.

CAT Enzyme Assays. CAT assays were performed as described (28). Protein extracts were prepared from tissues of hemizygous male mice, and 30 μg of total protein was incubated for 15 min to quantitate activity. The limit of detection of CAT activity was determined by assaying serial dilutions of commercially obtained CAT enzyme (Boehringer Mannheim) of known activity. Calculations of specific activity and conversion to molecules of enzyme were based on a native M_r of 80,000 (29) and a specific activity of 100,000 units/mg (Boehringer Mannheim), such that 1 unit of activity corresponded to 6.2×10^{10} molecules of active tetramer. One unit was defined as the conversion of 1 nmol of [^{14}C]chloramphenicol to the 3′-acetyl form in 1 min at 25°C.

Luciferase Enzyme Assays. Tissues were homogenized in a Dounce homogenizer in phosphate-buffered saline, pH 8.0, cellular debris was pelleted for 5 min at 12,000 × *g*, and supernatants were assayed immediately. Extracts (10 μg of total protein) were assayed in 100 mM Tris·HCl, pH 7.5/15 mM $MgSO_4$, 5 mM ATP; 1 mM D-luciferin was injected immediately before recording with a Monolight 2001 luminometer (Analytical Luminescence Laboratory, San Diego, CA). Peak light emission was measured, as it is both sensitive and linear over several orders of magnitude. Calculations of enzyme activity and number of luciferase molecules were determined by using an M_r of 60,000 (23). Specific activity and limit of detection measurements were determined based upon serial dilutions of a solution of crystallized firefly luciferase (Boehringer Mannheim) into testis extracts of C57BL6J/DBA2J nontransgenic mice. It was determined that 10^9 luminometer units (lu) were equivalent to 1 mg of active luciferase protein, and it was then calculated that one lu corresponds to 5×10^5 luciferase molecules.

RESULTS

Expression of Endogenous and Human *PGK2* in Transgenic Mice. To determine the pattern of expression of the human *PGK2* gene in transgenic mice, an 8-kb *Eco*RI genomic fragment was purified and injected into single-cell fertilized mouse embryos. This genomic fragment includes the entire *PGK2* coding sequence flanked by 2.5 kb upstream and 4.25 kb 3′ to the gene (Fig. 2*A*). Six founder mice were identified by hybridization to a human *PGK2* probe. Lines were established from three of the six founder mice, and each was subsequently analyzed.

Murine *Pgk-2* expression has been shown to be testis-specific (4, 6) and developmentally regulated (7, 11, 14). To determine the tissue specificity of the human *PGK2* transgene, total RNA was prepared from various tissues of adult males from each of the three transgenic lines (designated P4, P14, and P38), and expression of the transgene was analyzed using Northern (RNA) blots. Expression of the human *PGK2* RNA was testis-specific in all three lines tested. Line P14 was bred to obtain animals that were homozygous for human *PGK2*, and expression of the transgene in this line was followed (Fig. 3*A*). Testes from homozygous mice contained approximately twice the amount of transgene RNA per μg of total RNA as the hemizygous testis, consistent with regulation at the transcriptional level. Among the three transgenic

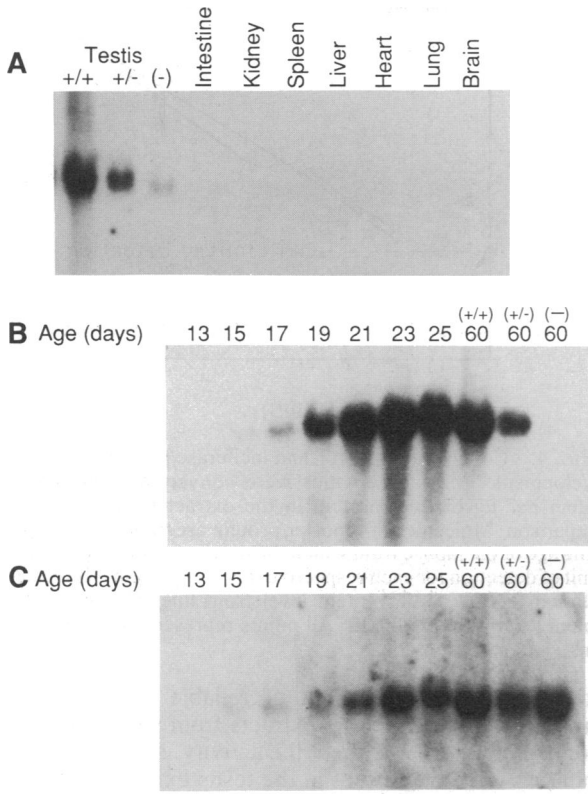

FIG. 3. Expression of *PGK2* in transgenic mice. (*A*) Northern analysis of various tissues from transgenic line P14. Total RNA (20 μg) from each of the tissues indicated was loaded per lane and probed with human *PGK2* sequences that share 70% homology to the mouse *Pgk-2* message. The faint band in the nontransgenic (−) testis lane is presumed to be crosshybridization to the endogenous mouse *Pgk-2* message. (*B*) Developmental expression of human *PGK2* in homozygous transgenic line P14. Total RNA was prepared from testes of line P14 mice sacrificed between day-11 and day-60 (date of birth = day-0). Twenty micrograms of total RNA per lane from the days indicated was loaded and hybridized to the same probe as in *A*. (*C*) Developmental expression of mouse *Pgk-2*. The filter in *B* was stripped and reprobed with mouse *Pgk-2* 3′ untranslated sequences. This probe is 50% homologous to the human *PGK2* sequence.

lines tested, however, there was no observed correlation between the number of integrated copies of the human gene and the level of human *PGK2* message (unpublished results, M.O.R.).

To determine the time during onset of spermatogenesis when the human *PGK2* transcript first appeared, RNA was prepared from testes of line P14 homozygous mice between day-11 and day-60. Transgene RNA was first detected at day-15 and increased to a high level by day-60 (Fig. 3*B*). Day-15 typically correlates with the appearance of pachytene spermatocytes (3). The presence of cytologically recognizable pachytene cells in this line at day-15 was confirmed by direct microscopic examination of dissociated testis cells. To determine whether the human transgene showed the same pattern of developmental expression as the endogenous mouse *Pgk-2* gene, the Northern blot was hybridized to a mouse 3′ specific probe at high stringency such that no crosshybridization to the human transgene was seen. The pattern of expression of mouse *Pgk-2* was similar to the pattern seen with the human probe (Fig. 3*C*). Therefore, it was concluded that expression of the human *PGK2* transgene is regulated in a manner similar to that of the endogenous *Pgk-2* gene.

Promoter Analysis of Human *PGK2*. Having established that the transgene functions in a tissue-specific and developmentally regulated manner, various deletions of the 5′ noncoding region were tested to map the cis-acting elements involved in the transcriptional regulation of *PGK2*. A 1.4-kb *Hin*dIII fragment containing the promoter region of *PGK2* that extends to, but does not include, the translation initiation codon, was inserted into a unique *Hin*dIII site in pSVOCAT (22). The resulting *PGK2*/CAT fusion gene places the CAT gene under the control of the sequences 5′ to the human *PGK2* coding region and uses the 3′ untranslated region of the simian virus 40 large tumor antigen gene to provide poly(A) addition sequences. This construct was then used to generate three different injection fragments, each containing the CAT gene with different lengths of the *PGK2* 5′ region. Fragment lengths of 1.4 kb, 515 bp, and 192 bp were generated by digesting the 5′ end of the fusion gene with *Xba* I, *Pst* I, or *Ava* II, respectively (Fig. 2*B*). The 192-bp site was chosen because it marked the 5′ end of the CAAT consensus sequence, which functions as a transcriptional activator in many cell types (18) and was, therefore, considered to be the boundary of the minimal promoter.

Expression of *PGK2*/CAT Transgenes. Thirty mice born from the 1.4-kb *PGK2*/CAT injections were analyzed using Southern blot; six founder mice were identified, and lines were derived from four of the founders (designated B4, B17, B22, and B29). Two lines of mice (PS13 and PS28) were established from the 515-bp *PGK2*/CAT construct, and three lines (AV25, AV36, and AV41) were made from the 192-bp *PGK2*/CAT fusion gene. Tissues of adult males from each of the nine lines were tested for CAT activity by thin-layer chromatography, and activity was measured using a scintillation counter (Table 1). Tissues assayed were brain, heart, lung, liver, kidney, intestine, muscle, and testis. Testis-specific expression of the transgene was seen in three lines of the 1.4-kb fragment construct (B17, B22, and B29), whereas line B4 did not express the transgene. Both transgenic lines containing the 515-bp *PGK2* fragment also had CAT activity restricted to the testes. By contrast, none of the three lines containing the 192-bp fragment constructs exhibited CAT activity in any tissue assayed. These results show that, although efficient tissue specific expression could be directed with 515 bp of upstream *PGK2* sequences, expression was eliminated by removing 323 bp between position −515 relative to the initiation codon and the minimal promoter elements that begin at −192 bp.

Table 1. CAT activity in testes of transgenic PGK-2/CAT lines

	1.4-kb promoter				515-bp promoter		192-bp promoter		
	B4	B17	B22	B29*	PS13	PS28	AV25	AV36	AV41
CAT activity†	<0.03	1.04	0.59	1.37	0.44	0.78	<0.03	<0.03	<0.03
Tissue expressing enzyme	None	T	T	T	T	T	None	None	None

T, testes. B4–AV41 are transgenic lines.
*Line B29 expressed the following CAT activity, in milliunits (mU) per μg, in testis: day-11, <0.03 mU; day-13, 0.05 mU; day-14, 0.08 mU; day-15, 0.15 mU; and day-16, 0.14 mU.
†Expressed as milliunits per μg.

To assay for the presence of regulatory elements between 1.4 kb and 515 bp that might affect the level of expression, CAT activity was quantitated for all lines of mice carrying these fragments. Constructs containing the 1.4-kb fragment had ≈2-fold greater mean activity than those with the 515-bp fragment (Table 1), a difference in level of activity that has been seen between lines of a single construct. Therefore, no cis-acting elements appear to exert any additional effect in the region between the 1.4-kb fragment and the 515-bp fragment.

The developmental regulation of the *PGK2*/CAT fusion genes was assayed by monitoring the appearance of CAT enzyme activity in extracts prepared from hemizygous testes of line B29 (1.4-kb fragment) at days 11, 13, 14, 15, and 16. CAT activity was first detected at a low level in extracts from day-13 testes (footnote to Table 1), indicating that *PGK2*/CAT transcript is present at day-13 and is likely to coincide with the first appearance of pachytene spermatocytes. Line PS28 (515-bp fragment) was also analyzed with respect to developmental appearance of CAT activity, and a similar pattern of expression was seen (data not shown). Previous experiments have shown that mouse PGK-2 protein first appears in spermatids (7, 12). However, CAT protein is present before the appearance of spermatids, and developmental Northern blot analysis of line B29 first detects *PGK2*/CAT message at day-15 (unpublished work, M.O.R.). This result shows that the transgene is not only transcriptionally active, but also competent for translation of the CAT reporter gene.

Luciferase Expression in the Testis. To increase sensitivity and to determine whether the promoter could function independently of the reporter gene, a parallel study was conducted with transgenic mice in which the 1.4-kb upstream region of *PGK2* was fused to the firefly luciferase gene. The 1.4-kb *PGK2* *Hin*dIII fragment was inserted into the unique *Hin*dIII site of pSVOAL (23), which contains the luciferase-coding region and simian virus 40 poly(A) addition sequences, and the resultant fusion gene (Fig. 2*C*) was isolated and injected into mouse embryos. Three founder mice were identified, and breeding lines (PL4, PL5, and PL12) were established for each.

Expression of the *PGK2*/luciferase fusion gene was examined by making protein extracts from various tissues of hemizygous adult males of each line and assaying each sample (10 μg of total protein) in a luminometer for luciferase activity (Table 2). In the presence of the substrate D-luciferin and ATP, luciferase emits a transient peak of yellow light with a decaying bioluminescence lasting several minutes (30). *PGK2*/luciferase expression, measured by luciferase-catalyzed peak photon production, was found to be testis-specific. Testis extracts prepared from lines PL4 and PL12 gave signals of 1400 and 1600 lu per μg, respectively, whereas all other tissues assayed (brain, heart, lung, liver, kidney, intestine, muscle, and ovary) gave background readings (<10 lu). Line PL5 did not express the transgene in any tissue assayed. To standardize the luciferase activity assay, aliquots of commercially prepared pure firefly luciferase were tested in the same luminometer. Peak light emissions in the linear range were recorded from 500 pg–50 fg; 50 fg corresponded to 10 lu, the limit of reliable detection in the luminometer. Adding testis extracts from wild-type mice to pure luciferase did not affect the activity, suggesting that no inhibitory effect is present in the testis extract.

Table 2. Luciferase activity in transgenic *PGK2*/luciferase lines

Transgenic line	PL4	PL5	PL12*
Luciferase activity†	1400	<10	1600
Tissue expressing enzyme	T	None	T

T, testes.
*Line PL12 expressed the following luciferase activity, in lu per μg of protein, in testis: day-9, <10 lu; day-12, <10 lu; day-13, 49 lu; and day-15, 149 lu.
†Expressed as lu per μg.

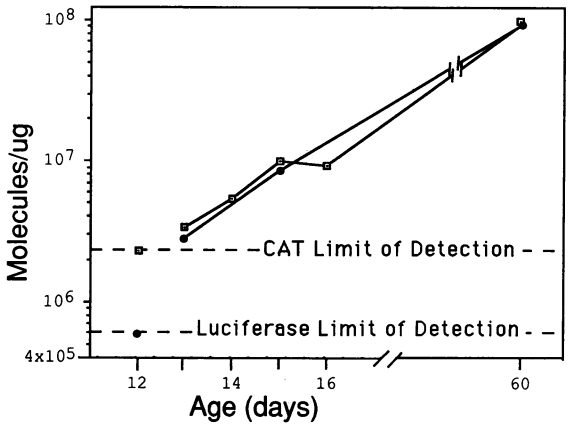

FIG. 4. Comparison of CAT and luciferase protein levels during development. Enzyme activities were converted to molecules of protein per μg of total protein in the extract to allow for direct comparison. Molecules of reporter protein are plotted as a function of the age of the mouse from which the protein extract was prepared. Limit of detection for each respective reporter protein is indicated by a dotted line. ⊡, CAT protein level from line B29; ●, luciferase protein level from line PL12. All points represent single determinations.

Developmental appearance of luciferase protein was examined for transgenic line PL12. Analysis of extracts of prepuberal testes from males of this line on days 9, 12, 13, and 15 revealed luciferase activity first appearing at day-13 (footnote to Table 2), consistent with the appearance of CAT activity in lines B29 and PS28. Apparently *PGK2*/luciferase message is also competent for translation in pachytene cells.

To directly compare the sensitivity and accumulation of each reporter enzyme, CAT and luciferase activities were converted to molecules of enzyme by using published and determined specific activities. Serial dilutions of commercially obtained CAT enzyme into nontransgenic testis extracts established the limit of detection at 0.03 milliunits, or 2×10^6 molecules of CAT tetramer. The limit of detection of luciferase enzyme was determined to be 10 lu or 5×10^5 molecules of luciferase protein, a 4-fold increase in sensitivity over CAT detection levels. Plotting the activities of reporter molecules in the transgenic lines B29 (CAT) and PL12 (luciferase) as a function of development (Fig. 4) demonstrates that the reporter genes function in a similar fashion with respect to accumulation of protein. Therefore, based on identical tissue specificity, developmental appearance, and accumulation kinetics, the control elements present in these constructs appear not to be affected by the sequences present in the reporter genes used.

DISCUSSION

Deletion analysis of the human *PGK2* gene in transgenic mice serves to define the extent of the sequences that encode transcriptional control elements. A 323-bp region between the functional 515-bp upstream region and the nonfunctional 192-bp sequence contains information essential for directing testis-specific and developmentally regulated expression. The 192-bp fragment contains sequence elements that are probably essential for promoter function—e.g., the CAAT box and GC box sequences (Fig. 1); however, these elements clearly require the upstream sequences to function. This fact is based on the observation that although none of the three transgenic lines containing the 192-bp *PGK2* fragment

showed any expression, 10 of 12 transgenic lines containing larger upstream regions exhibited testis-specific expression of the *PGK2* transgenes. Possibly elements located upstream of the 1.4-kb location normally exert effects on the levels of transcription of human *PGK2*; however, we have shown here that such elements are not essential for proper expression.

Another germ-cell-specific gene the control regions of which have been mapped is the mouse protamine 1-encoding gene (*Prm-1*) (31). Although *Prm-1* transcription initiates in round spermatids and not in pachytene cells, comparison of the regulatory regions of *Pgk-2* and *Prm-1* provides candidate sequence elements that may be involved in the control of tissue-specific expression. Both *Prm-1* and human *PGK2* 5′ regions contain two copies each of the sequence GGGTGGGG, present in one orientation in *PGK2* and in the opposite orientation in the *Prm-1* gene (20). It is also found in the upstream region of the *Prm-2* gene in the same orientation as *PGK2* (20). This sequence could have a role in tissue-specific gene expression; however, it should be noted that this sequence has not been identified in the available upstream sequence in the mouse *Pgk-2* gene.

A study of genes expressed at different times during spermatogenesis has resulted in the identification and cloning of several cDNAs corresponding to transcripts that display a pattern of expression nearly identical to that of *Pgk-2* (14). If *Pgk-2* is one of a number of coordinately expressed genes, then cis-acting elements responsible for the regulation of *Pgk-2* expression may be reiterated in the control regions of these other genes. Elucidation of common cis-acting regulatory elements would establish a mechanism for coordinate gene regulation at this time during spermatogenesis.

We have shown that the *PGK2*/CAT and the *PGK2*/luciferase fusion genes are translated concurrently with the first observable *PGK2* and *PGK2* fusion gene transcripts. Previous reports, however, suggest that translation of the endogenous message is delayed (7, 12). This difference can be the result of a number of effects. Possibly the *PGK2*/CAT fusion genes are missing regulatory elements required for translational control of the message, either because of species differences between human and mouse *Pgk-2* or because the fusion gene is lacking the coding region and the 3′ untranslated sequences of the *PGK2* message. Alternatively, it is possible that although *PGK2* and *PGK2* fusion gene messages are translationally controlled, a low rate of translation exists in pachytene cells that is only detectable by using CAT and luciferase reporter genes. PGK-2 protein, however, would not be observed until the increased translational efficiency in later cell types permitted detection.

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